INVESTIGATION OF THE ALKALOID CONTENT OF SOME SOUTH AMERICAN BARKS

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED INVESTIGATION OF THE ALKALOID CONTENT OF COME

SOUTH AMERICAN BARKS

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in Chemistry

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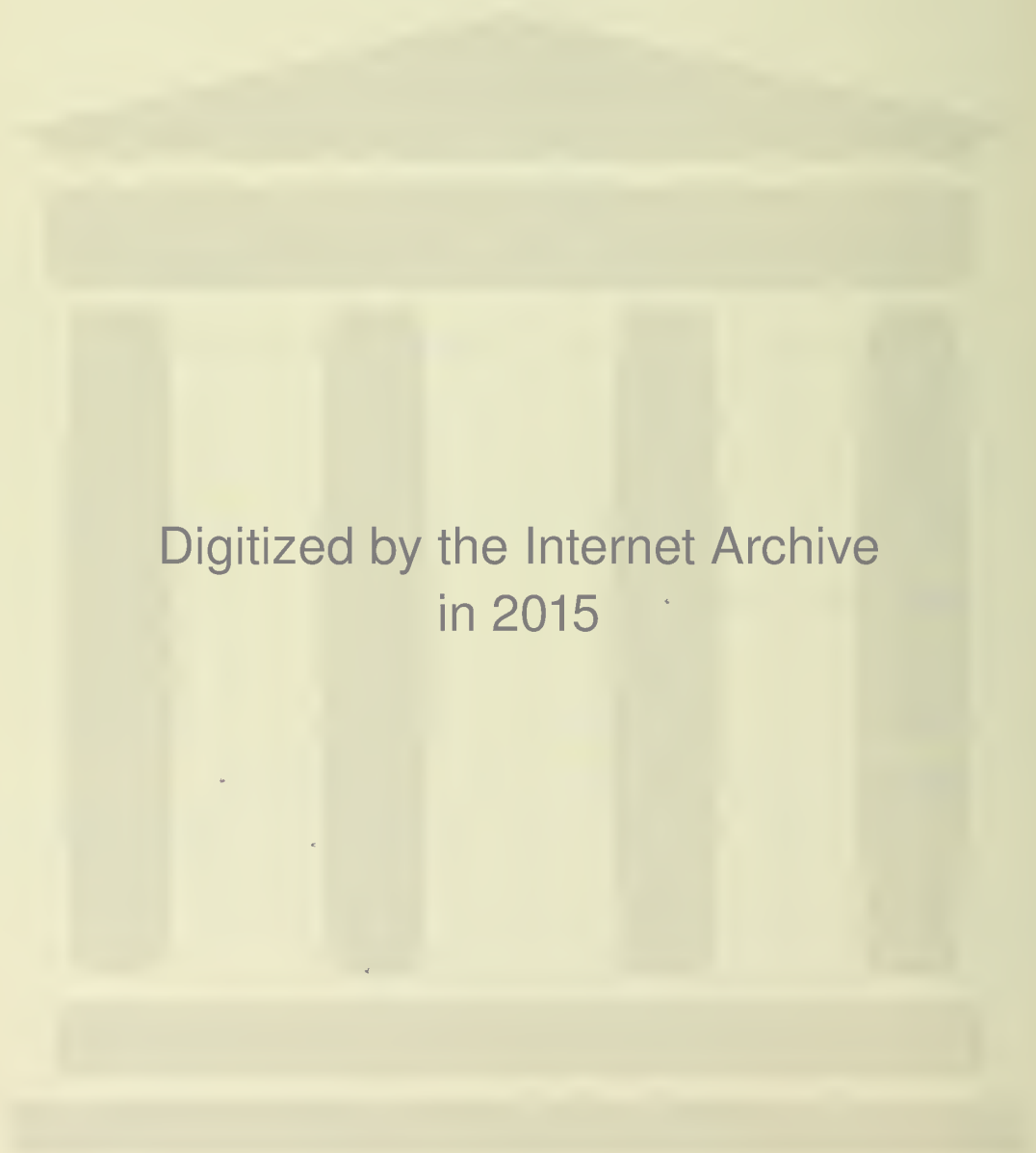
INTRODUCTION

The research work that I have done embraces a period of three semesters among which the required ten hours were divided. During this time I have worked with carbohydrates and herbs from Peru, South America. In the first case levulinic acid and furfural were studied and in the latter case Peruvian bark, saponin bark, and algarrobo beans were studied. The last part of my work which constitutes the isolation of the alkaloid or alkaloids in algarrobo, was investigated because of the physiological properties of the herb, as claimed by the natives of Peru who use it as a stimulant.

The work has not been completed. However, there have been obtained some results that should be of value for anybody interested in a further work with the same material.



THEORETICAL PART

Levulinic Acid

The yield of the acid was studied according to the methods of W.A. Noyes in his "Manual for Organic Chemistry", and Vanino's "Handbuch Der Praparativen Chemie". In the first method cane sugar was hydrolysed by hydrochloric acid and water into fructose and glucose f ollowed by their decomposition into levulinic and formic acids and water. In the second case potao starch was used and hydrolysed by a dilute solution of hydrochloric acid. Ihave found that W.A.Noyes' method gave a better yield than Vanino's.

Furfural

The Eastman Kodak Co. referred to the university that they could not obtain more than five percent of furfural from corn cobs. This was five percent less than the percent claimed by the university. For this reason three checks were run on the preparation of furfural according to the directions published by Dr. R. Adams, Dr. O. Kamm and Dr. C. S. Marvel in a bulletin entitled "Organic Chemical Reagents. The results were satisfactory inasmuch as nine and three tenths percent was obtained.

Algarrobo

This bean was identified by Dr. Trelease of the department of Botany as belonging to the Prosopis Horrida. It is a yellow bean about six to seven inches long, three eighths ofan ich wide and one fourth of an inch thick. It contains a dark brown seed similar to those of apples, and has a sweet smell. As stated in the introduction the bean was studied so as to determine the substance that had the stimulant property. It was supposed that the bean would con-

tain an alkaloid of very good physiological properties. With these ideas in mind, the work of investigation was undertaken. Although the results are not the best, an approximation to the proper method of extraction has been accomplished. Four methods of extraction were tried before anything of the alkaloid was seen.

EXPERIMENTAL PART

Levulinic Acid

A liter flask was used. In this flask one hundred cubic centimeters of water and one hundred grams of cane sugar were mixed and heated on the steam bath for twenty hours, with a condenser attached to the flask. After this time the mixture was filtered and the residue boiled with one hundred cubic centimeters of water and filtered also. To the combined filtrates thirty-five grams of sodium hydroxide were added in solution. This alkaline solution was evaporated to about one hundred cubic centimeters, filtered and extracted five times with fifty cubic centimeter portions of ether. The ether was distilled off. The residue was distilled under diminished pressure, and the fraction boiling at 140-160 degrees centigrade and twenty-five millimeters pressure was collected. The distillate was frozen in a wide mouth bottle. The watery residue was separated and the res warmed to room temperature and weighed. The first trial gave twelve grams of levulinic acid, and the second one seventeen.

In page 142 of Vanino's second volume, the following directions were found: Mix one liter of hydrochloric acid sp. gr. 1.1 with 1 kilogram of starch to a syrup and then reflux the mixture for twenty hours. Cool, filter, and distill under vacuum. The remainder

was purified in the same way as the preceeding one. By this method 138 grams of acid were obtained. The previous method gives a higher percent yield.

Furfural

As already stated the object of working with furfural was to check the yield of the substance from corn cobs. The process was as follows: In a 12 l. round bottom flask fitted with a cork stopper holding 500 cc separatory funnel and a condenser set downward for distillation, are placed 1300 grams of corn cobs (ground to about the size of corn kernels), 1800 cc of commercial concentrated hydrochloric acid, and 4600 cc of water. After standing for two to three hours, the flask was shaken, and heat applied. As the distillate comes over, more acid solution is added (1 l.of acid to 15 l. of water)The presence of furfural is shown by an immediate test of of a few drops of the distillate on a piece of paper containing some aniline acetate. This teste is very delicate. The distillat e is then treated with sodium hydroxide to slight acidi ty, and then salted and distilled until the distillate does not give a test for furfural with aniline acetate. The first portions coming over are collected until the liquid is uniform.

The wet furfural is now distilled under diminished pressure using an oil bath the temperature of which should not be allowed to rise above 130 degrees centigrade. The first distillate co sits of water and furfural but after about 60 cc have distilled, purefurfural distils over. It boils at 90 degrees centigrade at 65 mm. pressure. The yield of the first batch was 9.3 percent, and of the other runs 8 percent. The last two runas were partly spoiled.

Peruvian Bark

In order to determine the percent of quinine in the bark the following method was used: The bark was ground to almost a powder and to a poind of it ninety grams of lime in 900 cc of water were added. The mixture was stirred well and then dried in a steam bath. The dry mixture was then extract ed with 800 cc of chloroform for 12 hours. At the end of this period the chloroform was filtered and a fresh amount added, 600 cc. The combined extracts were shaken with dilute sulphuric acid, 225 cc. After separating the acid, another portion of 150 cc was added. The remaining chloroform solution was washed with water until no coloration was noticed. The combined water and acid solutions were neutralized by ammonium hydroxide, and evaporated until crystals began to form. At this point the solution was cooled, and the crystals separated. The remaining solution was once more evaporated and the crystals were sepated. In this way 3.85 percent of quinine was obtained. á sample directly imported from Peru was run through the same process, and 3.15 percent of cuinine was obtained.

A soxhlet extraction was run, which did not come out very well. It seems that the refluxing to which the chloroform is submitted affects the quinine somehow. The y ield increased by five tenths percent, but this result is not very sure because the melting point was not satisfactory.

Saponin Bark

The following was the method of extraction for saponin: Extract three times with water the ground bark, boiling for 5 hours each time. The combined extract was evaporated to dryness. The residue was ground and extracted with 80 percent alcohol. The alcohol

extraction was continued for four hours according to Stütz. However, although the percent of the crude saponin was found to be over 35 percent, the directions for its purification did not yield any amount saponin. Different samples were tried of 50 grams and 25 grams, but in each case no results were obtained. For 50 grams of extract 1000 cc of 80 percent alcohol were used.

In the first extraction the liquid was cooled before filtering, and a crystalline product was obtained, while in the second extraction the liquid was filtered while hot, and a gummy extract was obtained, which was hard to handle.

While the bark was boiling I noticed a white crystalline substance separating at the level of the liquid which seems to be pure saponin.

Algarrobo

After reading several general methods of analysis for herbs containing alkaloids, the following one was used. The algarrobo bean was ground as fine as possible (20 mesh), then it was extracted with ether in a soxhlet extractor for twenty hours. The ether solution had a light yellow color at the end of this time. Then the ether solution was shaken with three fifty cc portions of a 10 percent hydrochloric acid in a separatory funnel. The combined portions of acid were neutralized with ammonium hydroxide, and this alkaline solution was shaken with three 50 cc portions of ether and evaporated todryness. There was a very small residue, which was taken up by one cc of a five percent hydrochloric acid. The acid solution was afterward tested with Mayer's reagent. No precipitate was seen.

After this failure to obtain any alkaloid with an ether extraction, an alcohol extraction was tried. The ground bark was

placed in a percolator and percolated with three 150 cc portions of hot 92 percent alcohol. The combined extract solutions were filtered and evaporated to a thick syrup. Then the syrup was treated with a five percen hydrochloric acid, and left to stand for forty-eight hours to deposit resinous matter. The solution was filtered afterwards and because it was too dark in color, it was clarified with lead acetate. The lead was precipitated with hydrogen sulphide, filtered, and the hydrogen sulphide precipitation repeated two more times , in order to insure a total separation of the lead. To the clear solution ammonium hydroxide was added in excess. Here no precipitate was obtained, but instead a slight change in color.

The alkaline liquid was shaken in s separatory funnel, with four 50 cc portions of ether. 50 cc portions seemed to do an efficient extraction. The ether extract, which was clear, was run into a separatory funnel and left standing to separate water. It was finally dried with calcium chloride and evaporated under diminished pressure. The residue was taken up by 1.5 cc of water sligtly acidified with hydrochloric acid. One fifth of this small amount was tested with Mayer's reagent which gave a very good test for alkaloids. The precipitate, which was slightly yellow, turned brownish on standing. This was a proof of the presence of alkaloid in the Algarrobo bean. However, its solubility in alcohol seemed to be very little.

A water percolation was tried next. The water extract was run through the following process: It was made alkaline and then extracted in a separatory funnel with three 60 cc portions of ether. The ether was extracted with dilute hydrochloric acid. The acid solution was made alkaline with ammonium hydroxide and then it was

extracted with ether. The ether solution which was supposed to contain the alkaloid, was evaporated to dryness. The residue was taken up by four cc of acid and a fraction tested with Mayer's reagent. The test was very good, and it revealed that the alkaloidwas more soluble in water than in alcohol. Theis not only because of the amount of precipitate but also by the amount of residue left after the ether was evaporated to dryness.

Another process was then tried. 150 grams of ground bean were treated with a solution of 10 grams of lime and one hundred cc of water. The mixture was stirred well, dried in a steam bath, reground, and extracted with ether in a soxhlet extractor for twenty hours. The ether extraction was treated as in the first trial. After the ether was dried and distilled under diminished pressure, an oily residue was left,about 0.7 cc, and about three white crystals of the size of a sugar granule were also found. It was questioned whether this oily liquid could be some fat extracted from the bark. The amounts were so small that no constants could be investigated. As a conclusion, it can be said that there is alkaloid in the bran, but that the percent is very small, also that the alkaloid is in a form more soluble in water than in any other solvent, and that only the lime treatment renders it soluble in ether.

In as much as the bean was very sweet an analysis to determine the kind of sugar was made. An osazone of the unknown sugar was prepared which melted at the melting point of maltose. For this reason the osazone of pure maltose was prepared, its melting point taken, and also mixed melting points. All the melting points werethe same. This indicated that maltose was the sugar in the bean. Besides, a microscopic examination, where the crystals of the osazone of the

bean corresponded to pictures of the crystals of pure maltosazone.

SUMMARY

Summing up all of the work, it has been found that in the case of furfural the distillate from the flask where the corn cobs and the acid solution boils, always gives a test for furfural irrespective of the time of distillation. This seems to indicate that the process can be improved, in order to obtain a larger percent of furfural.

Peruvian bark is an herb that has been studied, and, in my case, I only worked on the percent of quinine in the vark dirctly imported from Peru. In spite of the fact that the soxhlet ectraction of the bark was not satisfactory, I believe that a similar process with slight qualifications as to temperature and air exposure of the apparatus, so as not to have to heat the chloroform so much, can be divised. If th chloroform could be boiled under diminished pressure while subject to the soxhlet extraction, this would prevent any chemical action during the extraction, for this would render a lower boiling point.

Regarding saponin, not much work was done with it except to follow the method outlined by Sütz. However, there is a field of study in the purification of the saponin.

In the case of algarrobo, Ihave given a detailed explanation of the process and results above. I just wish here to restate the fact that the process of extraction of the alkaloid or alkaloids can be improved in orderto obtain a larger yield. A further study of this bark would reveal important things, because the only man who worked with it in Peru, although he did not carry a chemical analysis prepared a beverage which he labled "Algarrobina", and which had a

very wood tonic effect. This medicine, due to the crude way in which it was prepared, and due as stated, to the lack of more knowledge of the chemical character of the active substace, does not have a large market. It was my intention to furnich a complete chemical analysis of the bark and further more, to study the physiological activity of the alkaloid. However, Ihave not accomplished these aims entirely, but in part. Because the percent is very low, it can be inferred that the alkaloid must indeed be very active.

The seeds do not contain any alkaloid.

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